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RESEARCH ARTICLE

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Cobalt chloride exposure dose-dependently induced hepatotoxicity through enhancement of cyclooxygenase-2 (COX-2)/B-cell associated protein X (BAX) signaling and genotoxicity in Wistar rats

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Abstract

Cobalt chloride ($CoCl_2$) is one of the many environmental contaminants, used in numerous industrial sectors. It is a pollutant with deadly toxicological consequences both in developing and developed countries. We investigated toxicological impact of $CoCl_2$ on hepatic antioxidant status, apoptosis, and genotoxicity. Forty Wistar rats were divided into four groups, 10 rats per group: Group 1 served as control and received clean tap water orally; Group 2 received $CoCl_2$ solution (150 mg/L); Group 3 received $CoCl_2$ solution (300 mg/L); and Group 4 received $CoCl_2$ (600 mg/L) in drinking water for 7 days, respectively. Exposure of rats to $CoCl_2$ led to a significant decline in hepatic antioxidant enzymes together with significant increase in markers of oxidative stress. Immunohistochemistry revealed dose-dependent increase in cyclooxygenase-2 and BAX expressions together with increased frequency of Micronucleated Polychromatic Erythrocytes. Combining all, $CoCl_2$ administration led to hepatic damage through induction of oxidative stress, inflammation, and apoptosis.

KEYWORDS

apoptosis, cobalt chloride, genotoxicity, micronucleus test, risk assessment, Wistar rats

1 | INTRODUCTION

Cobalt (Co) emission increased significantly during the twentieth century and it is present in the atmospheric, aquatic, and terrestrial environments. Also, it is one of the most toxic environmental pollutants and human exposure is of great concern[1]. From the onset, the use of Cobalt chloride ($CoCl_2$) had been reported with toxic effects such as thyroid dysfunction in children and various cardiovascular conditions in heavy beer drinkers.[2] It has also been documented for use as a blood doping agent in humans and animals,[3] and together with medical importance as Co-containing hip implants[4,5] for surgical patients. Reference 6 reported the hepatotoxicity associated with $CoCl_2$ toxicity during late pregnancy and early postnatal period. From our laboratory we have reported hypotensive[7,8] and hypertensive[9] effects of $CoCl_2$ in different dosages and at different experimental set-ups. Similarly, Ref. 10 has reported positive correlation between reduced serum nitric oxide and $CoCl_2$ intoxication. Furthermore, the cytotoxicity and inflammation induced by $CoCl_2$ has been reported through the inhibition of hypoxia inducible factor.[11]

A minute amount of reactive oxygen species (ROS) is necessary for normal metabolic processes since it plays various regulatory roles in cells metabolism.[12] The hepatic cells are equipped with an effective and complex antioxidant system against oxidative stress, including protective enzymes and biological antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and glutathione-s-transaminase (GST).[13,14] To our knowledge, only one study has been carried out on the cobalt chloride induced hepatotoxicity in adult female rats and their suckling pups.[6]

Oxidative stress, inflammation, and apoptosis have been implicated in the mechanisms of action of $CoCl_2$ toxicity.[15] Oxidative stress is an imbalance between ROS and antioxidant defense mechanism.[16] Cobalt ion has the ability to participate in Fenton reaction and trigger the production of ROS, therefore causing oxidative modification of lipid, protein, and DNA of cells.[17,18] The present study was aimed at investigating hepatic damage that might result from indiscriminate use of $CoCl_2$ and its toxicological impact on hepatic antioxidant status, apoptosis, and genotoxicity and the molecular mechanism of action.

2 | MATERIALS AND METHOD

2.1 | Chemicals

Cobalt chloride ($CoCl_2$; CAS Number (7791-13-1) and percentage purity (≥97%), cation traces: (Fe: ≤0.005%), and anion traces: nitrate (NO_3^-): ≤0.01%, sulfate (SO_4^{2-}): ≤0.007%.), Potassium hydroxide, reduced glutathione (GSH), Trichloroacetic acid, sodium hydroxide, 1, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), xylenol orange, and hydrogen peroxide (H_2O_2), N-(1-naphthyl) ethylenediamine dihydrochloride, cyclophosphamide, 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma (St Louis, MO, USA). Normal goat serum, Biotinylated antibody and Horse Radish Peroxidase (HRP) System was purchased from (KPL, Inc., Gaithersburg, Maryland, USA). COX-2 and BAX antibodies were purchased from (Bioss Inc. Woburn, Massachusetts, USA) while 3,3′-Diaminobenzidine (DAB) tablets were purchased from (AMRESCO LLC. OHio, USA). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

2.2 | Animal care and procedures

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.[19]

Forty male albino rats were used in this study. The rat weights ranged between 140 and 160 g. The rats were initially acclimatized for a period of 3 weeks after their purchase. The rats were housed in plastic cages with a 12-h light/dark cycle in a well-ventilated rat house. They were provided rat pellets with unlimited supply of water throughout the period of acclimatization and administration of the test compounds. The rats were randomly distributed into four groups of 10 animals each as follows; Group A, control group were given water for 7 days; Group B, received 150 mg/L $CoCl_2$ alone in drinking water for 7 days; Group C, received 300 mg/L $CoCl_2$ in drinking water for 7 days; and Group D, received 600 mg/L $CoCl_2$ in drinking water for 7 days. Fresh $CoCl_2$ solution was provided daily. This study was carried out based on our previous work from our Laboratory as reported by Refs. 3,4.

2.3 | Preparation of microsomal fraction from liver tissues

After 7 days of $CoCl_2$ administration, the body weight of each rats were recorded. The rats were humanely sacrificed by cervical dislocation. The liver was harvested, rinsed in 1.15% KCl and homogenized in potassium phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 rpm for 20 minutes to obtain the post microsomal fractions (PMFs). The supernatants obtained were stored at −4°C until the time of use.

2.4 | Homogenization

The liver tissues were blotted with filter paper and weighed. The tissues for biochemical assays were homogenized in four volumes of the homogenizing buffer (0.1 M Phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenizing buffer was prepared by dissolving 0.496 g of di-potassium hydrogen orthophosphate (K_2HPO_4) and 0.973 g of potassium di-hydrogen orthophosphate (KH_2PO_4) in 90 mL of distilled water. The pH was adjusted to 7.4 and then made up to a 100 mL with distilled water. The resulting homogenate was centrifuged at 10,000 rpm for 10 minutes in a cold centrifuge (4°C), to obtain the post microsomal fraction. The supernatant was collected and used for biochemical analyses.

2.5 | Hepatic biochemical assays

The supernatants from the liver tissues were used for the following biochemical assays.

Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 7.2 at 30°C as described by Misra and Fridovich[20] with slight modification from our laboratory.[21] Briefly, 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. This preparation prevents oxidation of epinephrine. About 30 μL of liver PMF was added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 μL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome. The Catalase (CAT) activity was determined according to the method of Shinha.[22] Reduced GSH was determined at 412 nm using the method described by Ref. 23. Glutathione-S-transferase (GST) was estimated by the method of Ref. 24 using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Protein concentration was determined by the method of Ref. 25. The malondialdehyde (MDA) level was measured according to the method of Ref. 26. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$. Glutathione peroxidase activity (GPx) was measured according to Ref. 27. Hydrogen peroxide (H_2O_2) generation was determined as described by Ref. 28. Nitric oxide content was determined according to the method of Olaleye et al.[29] All the readings were done in triplicates.

TABLE 1 Markers of oxidative stress following exposure to $CoCl_2$

Groups	GROUP A (Control)	GROUP B (150 mg/L)	GROUP C (300 mg/L)	GROUP D (600 mg/L)
AOPP	0.15 ± 0.02	0.18 ± 0.04	0.21 ± 0.04[a]	0.23 ± 0.03[a]
H_2O_2	35.46 ± 3.54	42.24 ± 1.39[a]	44.53 ± 2.73[a]	44.32 ± 2.64[a]
MDA	7.06 ± 0.39	7.55 ± 0.69	14.11 ± 1.47[a]	13.04 ± 2.51[a]
Nitric oxide	4.64 ± 0.36	4.67 ± 0.13	4.8 ± 0.21	4.87 ± 0.16[a]

Values are presented as mean ± standard deviation ($n = 10$). Superscript (a) indicates statistically significant when groups B, C and D are compared with group A. Group A (Control), Group B (150 mg/L of $CoCl_2$), Group C (300 mg/L of $CoCl_2$), and Group D (600 mg/L of $CoCl_2$).
AOPP, advanced oxidation protein product (units/mg protein); H_2O_2, hydrogen peroxide (μmole H_2O_2 consumed/min/mg protein); MDA, malondialdehyde (micromole/mg protein); nitric oxide (units/mg protein).

2.6 | *In vivo* micronucleus assay technique

The proximal ends of the femurs were carefully removed with a pair of scissors until a small opening to the marrow became visible. The femur was submerged in fetal calf serum and the marrow was flushed out gently by aspiration and flushing on glass slides. The marrow suspension was positioned on one end of a slide and spread by pulling the material behind polished cover glass held at an angle of 45°. Slides were fixed in methanol for 3-5 minutes, allowed to dry for 24 hours and later stained with May–Gruenwald followed by 5% diluted Giemsa solution for at least 30 minutes. Slides were then rinsed in phosphate buffer for about 30 seconds and in distilled water and air-dried. These stained slides were mounted in DPX with cover slips. They were later viewed under the microscope at 100× magnification using oil immersion for the presence of micronucleated polychromatic erythrocytes (MnPCE). Scoring was done using a tally counter.

2.7 | Immunohistochemistry of liver COX 2 and BAX

The paraffin-embedded liver tissues were placed on charged slides and then dewaxed by immersion in xylene for 5 minutes (twice). It was then rehydrated in ethanol of 100%, 90%, and 80% concentrations for 5 minutes each. The slides were placed in distilled water tank for 5 minutes before incubating with endogenous peroxidase for 10 minutes. After incubation, they were rinsed with water and placed in wash buffer tank for 5 minutes and then rinsed with distilled water. Antigen retrieval was done by boiling in citrate buffer pH 6.0 and allowed to cool. Slides were then rinsed and placed in distilled water tank for 2 minutes. The sections were blot dried, and goat serum (KPL, Inc., Gaithersburg, Maryland, USA) added followed by incubation in a humidifying chamber for 15 minutes. After the incubation, the slides were shaken to remove excess goat serum, and incubated with COX-2 and BAX (1: 200; Bioss Inc. Woburn, Massachusetts, USA), respectively, at 4°C overnight in a humidifying chamber. The slides were rinsed with wash buffer and placed in wash buffer tank for 5 minutes. Biotinylated antibody (KPL, Inc., Gaithersburg, Maryland, USA) was added and sections were incubated in humidifying chamber for 30 minutes. Then, they were rinsed and placed in wash buffer tank for 5 minutes. The slides were removed, shaken and incubated with streptavidin HRP system (KPL, Inc., Gaithersburg, Maryland, USA) for 30 minutes. They were later rinsed and placed in phosphate buffer saline tank for 5 minutes. Slides were shaken and DAB (AMRESCO LLC., OH, USA) was added for 3 minutes. The reaction was stopped by rinsing with distilled water. The sections were counter-stained with HIGHDEF® IHC hematoxylin (Enzo Life Sciences, NY, USA) for 3 seconds. The slides were then transferred to 80%, 90%, and 100% ethanol for 3 minutes each after which they were transferred to xylene (100%) tank for 5 minutes (twice). The immunoreactive positive expression of COX-2 and BAX were observed on each slide under 100× magnification with a digital microscope.

2.8 | Histopathology

Small pieces of liver tissues were collected in 10% buffered formalin (pH 7.4) for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 μm in thickness were sectioned and stained with hematoxylin and eosin for histopathological examination.[30]

2.9 | Statistical analysis

All values are expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's *t* test and One-way analysis of variance (ANOVA) followed by Turkey post-test

TABLE 2 Hepatic glutathione content and markers of oxidative stress following exposure to $CoCl_2$)

Groups	GROUP A (Control)	GROUP B (150 mg/L)	GROUP C (300 mg/L)	GROUP D (600 mg/L)
GSH	189.46 ± 10.98	179.90 ± 9.26[a]	130.90 ± 5.00[a]	115.49 ± 9.63[a]
Total THIOL	0.29 ± 0.02	0.29 ± 0.04	0.32 ± 0.42[a]	0.34 ± 0.04[a]
Non Protein Thiol	0.50 ± 0.07	0.41 ± 0.07[a]	0.36 ± 0.21[a]	0.07 ± 0.02[a]

Values are presented as mean ± standard deviation ($n = 10$). Superscript (a) indicates statistically significant when groups B, C and D are compared with group A. Group A (Control), Group B (150 mg/L of $CoCl_2$), Group C (300 mg/L of $CoCl_2$), and Group D (600 mg/L of $CoCl_2$).
GSH (Reduced Glutathione; micromole/mg protein); Total Thiol (nmole/mg protein); Non Protein Thiol (NPT) (nmole/mg protein).

TABLE 3 Hepatic antioxidant enzymes status in rats following exposure to $CoCl_2$

Groups	GROUP A (Control)	GROUP B 150 mg/L)	GROUP C (300 mg/L)	GROUP D (600 mg/L)
GPx	76.21 ± 6.59	47.88 ± 5.22[a]	64.98 ± 3.48[a]	60.04 ± 5.70[a]
GST	3.28 ± 0.96	2.19 ± 0.70[a]	0.58 ± 0.83[a]	0.72 ± 0.16[a]
SOD	1.96 ± 0.01	1.93 ± 0.01	1.95 ± 0.02[a]	1.94 ± 0.02[a]
CAT	376.46 ± 8.33	327.38 ± 4.84[a]	320.50 ± 8.35[a]	335.75 ± 5.63[a]

Values are presented as mean ± standard deviation ($n = 10$). Superscript (a) indicates statistically significant when groups B, C, and D are compared with group A. Group A (Control), Group B (150 mg/L of $CoCl_2$), Group C (300 mg/L of $CoCl_2$), and Group D (600 mg/L of $CoCl_2$).
GPx, glutathione peroxidase (units/mg protein); GST, glutathione-S-transferase, *mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein*; SOD, superoxide dismutase, units/mg protein; CAT, catalase; mmole H_2O_2 consumed/min/mg protein.

using Graph Pad Prism version 5.00. Differences were considered significant at $P < .05$.

3 | RESULTS

3.1 | Hepatic markers of oxidative stress

The results obtained show that $CoCl_2$ treatment significantly $P < 0.05$ increased hepatic H_2O_2 generation, malondialdehyde (MDA) content and advanced oxidative protein product (AOPP) levels and nitric oxide (NO) contents in a dose-dependent fashion (Table 1). However, Non Protein Thiol (NPT) decreased $P < 0.05$ significantly while Total thiol (TT) $P < 0.05$ increased significantly (Table 2). Further, the hepatic activity of SOD in $CoCl_2$ treated rats were significantly $P < 0.05$ reduced when compared with the control (Table 2). On the other hand, the hepatic CAT activity was markedly $P < 0.05$ increased while the activities of GST, GPx, and GSH content declined significantly (Table 3) in $CoCl_2$ treated rats relative to the control. However, there were significant $P < 0.05$ increases in serum ALT and AST whereas serum total protein and albumin levels declined $P < 0.05$ significantly in $CoCl_2$ treated rats when compared with the control (Table 4). Interestingly, rats that received 600 mg/L of $CoCl_2$ had the lowest serum ALT levels. The micronucleus assay showed dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes (MnPCE) in $CoCl_2$ treated groups compared with the control (Figure 1).

3.2 | Histopathology and immunohistochemistry

Histopathology results showed that control group had no visible lesions while rats treated with $CoCl_2$ showed hepatocytes with focal areas of moderate congestion of vessels as well as very mild infiltration by inflammatory cells, focal area of necrosis and congestion of vessels (Figure 2). Immunohistochemistry of rats treated with $CoCl_2$ showed higher hepatic expressions of COX-2 and BAX in increasing doses relative to the control in a dose dependent manner (Figures 3 and 4).

4 | DISCUSSION

The current study has shown that $CoCl_2$ induced liver injury in rats. Oxidative stress has been documented to be involved in $CoCl_2$-induced toxicity[1]. Excessive production of ROS causes an imbalance between the antioxidant defense system and ROS levels in favor of ROS production which in turn leads to oxidative stress, inflammation and cell death.

Hepatotoxicity induced by $CoCl_2$ has been shown to manifest mainly as oxidative stress damage following generation of ROS and the depletion of antioxidant defense mechanism in the liver.[6,31] In this study, administration of $CoCl_2$ for 1 week resulted in a significant increase in MDA content, H_2O_2 generated, AOPP confirming earlier report[2]. The increase in the hepatic AOPP and NO indicated inflammation and this together also contributed significantly to oxidative stress.

Aspartate aminotranferase (AST) and Alanine Aminotransferase (ALT) are reliable determinants of liver parenchymal injury. The increase in the activities of AST and ALT in serum after hepatic injury might be due to the leakage of these enzymes from the liver cytosol into the blood stream. In the present study, $CoCl_2$ dose-dependently increased the serum ALT and AST levels which was indicative of hepatic damage. Interestingly, however, the rats that received the highest dose of $CoCl_2$ (600 mg/L) had the lowest serum ALT. It has been documented that several risk factors can decrease serum ALT activity and this might also contribute to the increase in the AST/ALT ratio.[32]

TABLE 4 Hepatic antioxidant enzymes status in rats following exposure to $CoCl_2$

Groups	GROUP A (Control)	GROUP B (150 mg/L)	GROUP C (300 mg/L)	GROUP D (600 mg/L)
ALT (IU/L)	19.82 ± 2.22	23.07 ± 2.95[a]	23.10 ± 4.38[a]	10.18 ± 0.29[a]
AST (IU/L)	24.35 ± 4041	24.79 ± 8.68	25.51 ± 0.66	28.55 ± 2.76[a]
ALBUMIN (IU/L)	1.00 ± 0.27	0.49 ± 0.09[a]	0.70 ± 0.07[a]	0.53 ± 0.03[a]
Total protein (mg/dL)	2.40 ± 0.12	2.36 ± 0.08	2.07 ± 0.15[a]	2.09 ± 0.19[a]

Values are presented as mean ± standard deviation ($n = 10$). Superscript (a) indicates statistically significant when groups B, C and D are compared with group A. Group A (Control), Group B (150 mg/L of $CoCl_2$), Group C (300 mg/L of $CoCl_2$), and Group D (600 mg/L of $CoCl_2$).

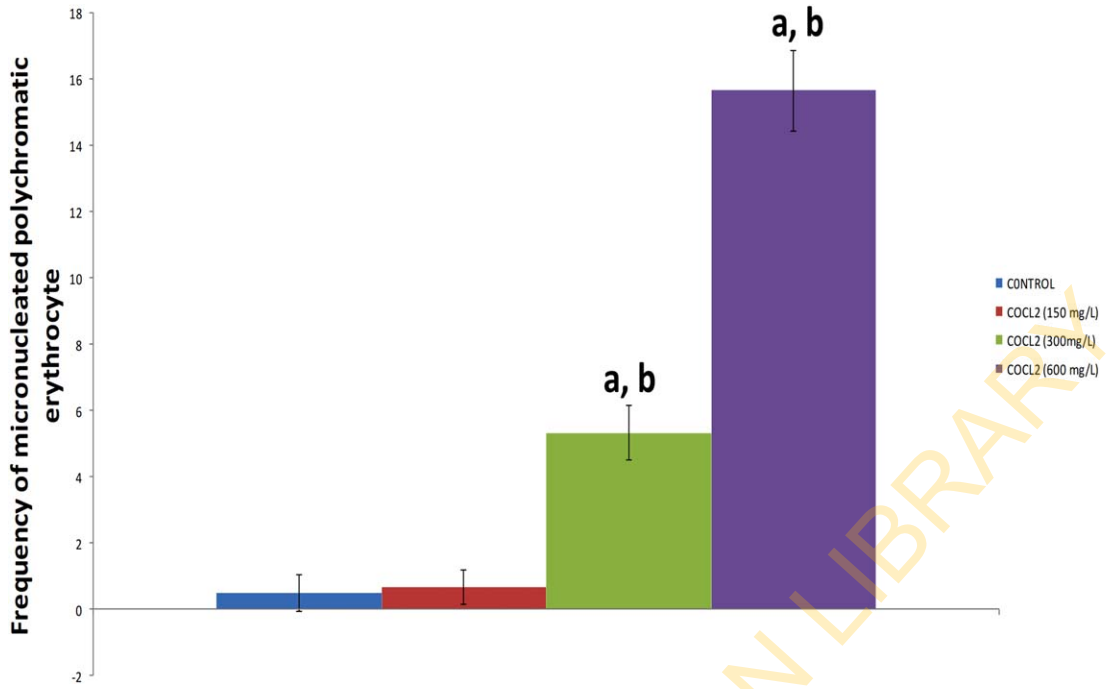

FIGURE 1 The effect of $CoCl_2$ on bone marrow of Albino rats. Data are presented as mean ± standard deviation. Superscripts (a) indicates significant difference ($P < .05$) when compared with control. [Color figure can be viewed at wileyonlinelibrary.com]

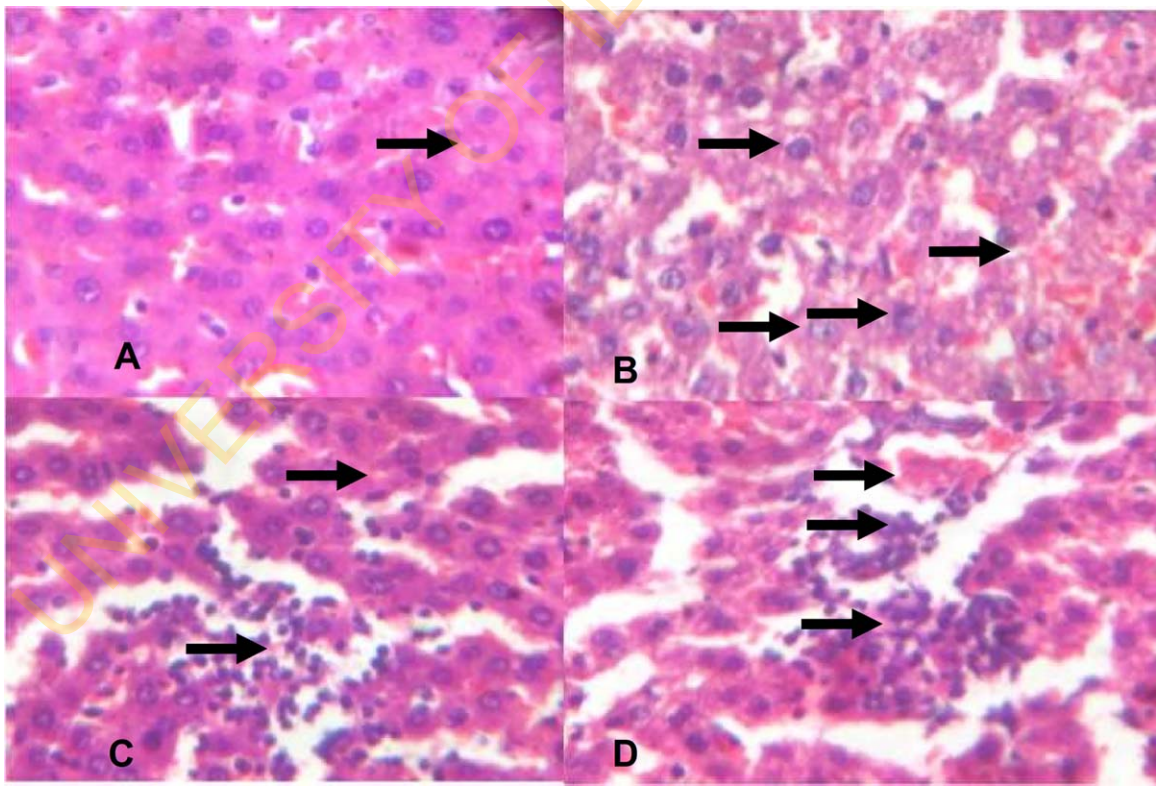

FIGURE 2 The effect of $CoCl_2$ on hepatic tissue. Group B (150 mg/L of $CoCl_2$) shows no significant lesion. Group C (300 mg/L of $CoCl_2$) little infiltration of inflammatory cells whereas the rats in Group D (600 mg/L of $CoCl_2$), show mild infiltration of the hepatic interstitium by inflammatory cells (black arrows). Histologic slides were stained with Hematoxylin and Eosin (magnification 100×). Abbreviation: $CoCl_2$, cobalt chloride. [Color figure can be viewed at wileyonlinelibrary.com]

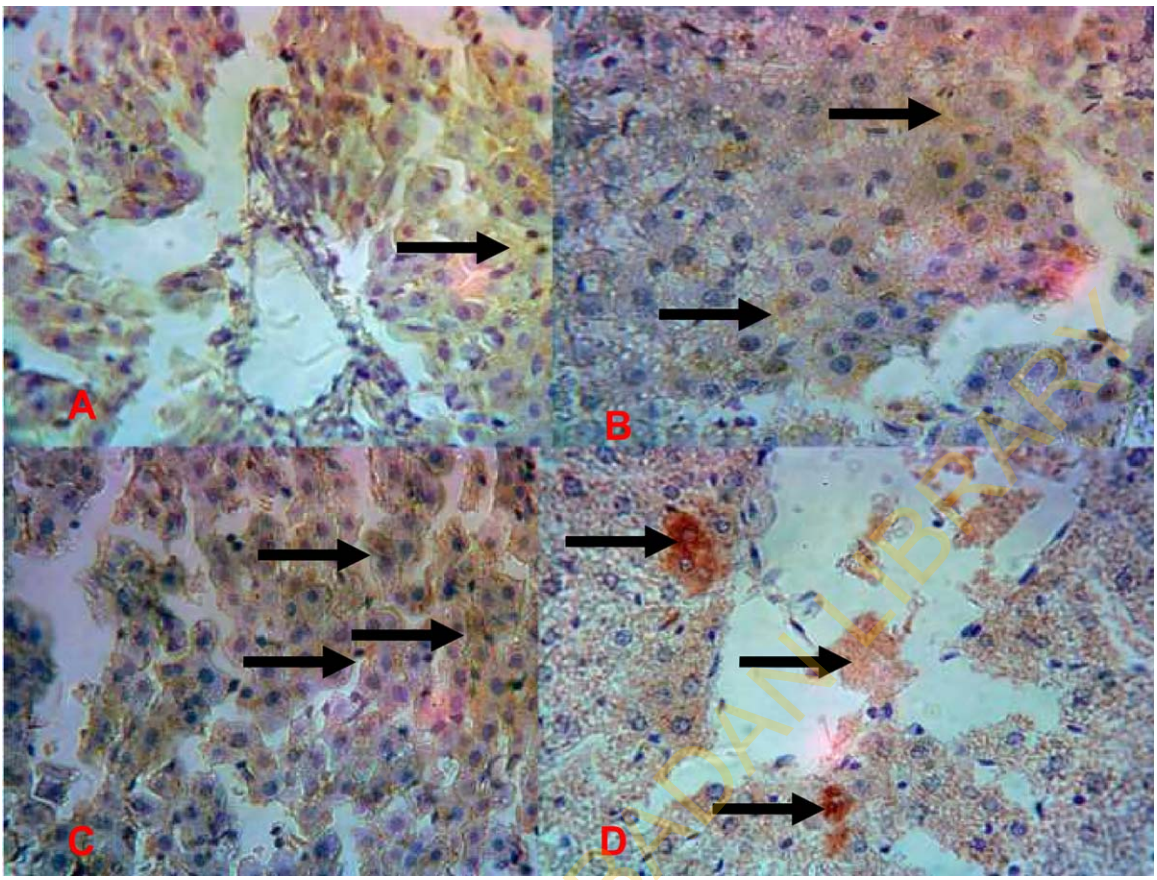

FIGURE 3 The effect of $CoCl_2$ intoxication of hepatic BAX expressions. Group A (control) shows lower expressions of BAX in the hepatic tissues. Group B (150 mg/L of $CoCl_2$) shows higher expressions of BAX. Group C (300 mg/L of $CoCl_2$) higher expressions of BAX than Groups A and B cells whereas the rats in Group D (600 mg/L of $CoCl_2$), show expressions of BAX higher than that of Group C (black arrows). The slides were counterstained with high definition hematoxylin and viewed 400× objectives (magnification 100×). [Color figure can be viewed at wileyonlinelibrary.com]

GSH is a thiol-containing intracellular antioxidant and is considered to be the redox buffer of cells as it helps to protect cells against oxidative stress and ROS.[33,34] Under normal physiological conditions, it is known that intracellular antioxidant enzymes, such as SOD, CAT, GST, and GPx, eliminate ROS and thereby playing an important role as an antioxidative agent against cellular damage.[35] Moreover, SOD catalyses the conversion of superoxide anion radicals to H_2O_2 in cells, thereby detoxifying H_2O_2, while CAT converts H_2O_2 into water as a second line of defense.[36,37] Also, GPx and GST as antioxidant enzymes work together with GSH as a co-factor in the breakdown of H_2O_2 to water.[38] In this study, the SOD, CAT, GST, and GPx activities were significantly decreased in $CoCl_2$ administered groups.

The cyclooxygenase 2 (COX-2) represents a key enzyme in arachidonic acid metabolism in health and disease.[39] The products of COX-2 activity (e.g., PGE2 and prostacyclin) have been shown to participate in diverse physiological and pathophysiological processes, including squamous cell carcinoma of the urinary bladder, liver, and colorectal tumorigenesis.[39,40] BAX is a central cell death regulator and a major proapoptotic member of the B-cell lymphoma 2 (Bcl-2) family proteins that control apoptosis in normal and cancer cells.[41] BAX activation has been reported to induce mitochondrial membrane permeability, thereby leading to the release of apoptotic factor cytochrome c and consequently cancer cell death.[41–43]

From the preset study, administration of $CoCl_2$ increased the expressions of COX-2 and BAX together with observable increase in the frequency of MnPCE. It is worth to note from the present study that administration of $CoCl_2$ enhanced oxidative stress, inflammation and apoptosis. Together, this might have potentially contributed to the genotoxic effect of $CoCl_2$. The involvement of oxidative stress, inflammation, and apoptosis and their relationship with genotoxicity has been reported elsewhere.[44–46] However, prolonged oxidative stress can lead to chronic inflammation which in turn might serve as a link to some chronic diseases such as cancer, diabetes and cardiovascular diseases. The underlying mechanism involves in the activation of a variety of transcription factors such as NF-κB, PPAR-γ, p53, and NRF2 by oxidative stress ultimately leads to expressions of various genes including inflammatory cytokines, chemokines and anti-inflammatory molecules.[47] Hence, we propose that the increased frequency of MnPCE was associated with the interaction and enhancement of oxidative stress, inflammation, and apoptosis.

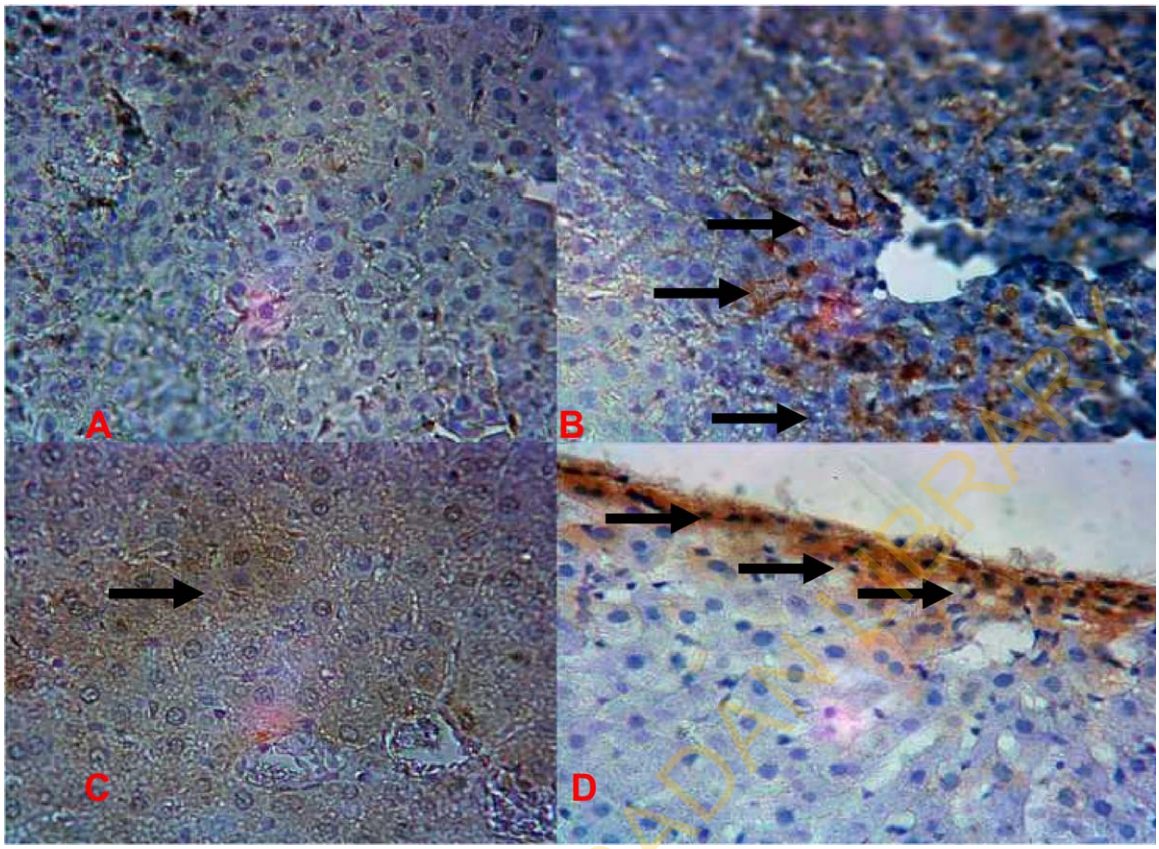

FIGURE 4 The effect of $CoCl_2$ intoxication of hepatic cyclooxygenase 2 (COX-2) expressions. Group A (control) shows lower expressions of COX-2 in the hepatic tissues. Group B (150 mg/L of $CoCl_2$) shows higher expressions of COX-2. Group C (300 mg/L of $CoCl_2$) higher expressions of COX-2 than Groups A and B cells whereas the rats in Group D (600 mg/L of $CoCl_2$), show expressions of COX-2 higher than that of Group C (black arrows). The slides were counterstained with high definition hematoxylin and viewed 400× objectives (magnification 100×). [Color figure can be viewed at wileyonlinelibrary.com]

Oxidative stress and inflammation are closely related pathophysiological processes that can easily be induced by various stimuli including cigarette smoke.[48] Inflammation involves the actions of inflammatory cells causing increased uptake of oxygen together with production and accumulation of ROS. Inflammatory cells produce soluble mediators that act by recruiting other inflammatory cells to the site of inflammation and sustenance of the inflammatory-oxidative environment.[49] This environment can cause injury to neighboring healthy cells and tissues over a period of time. Oxidative stress has also been linked to apoptosis via activation of various signal transduction pathways (Janus kinase/signal transducers) by cytokines and various biological effects including proliferation and immune response of which inflammation is an example.[50]

Together, we hypothesized that the observed hepatotoxicity in the present study might be associated with oxidative stress, free radical generation, inflammation, and reduction in the hepatic antioxidant defense system. Also, caution must be taken into consideration in using $CoCl_2$ as trace element in animal production. Hence, we report for the first time the hepatotoxic effect of $CoCl_2$ and its mechanism of action toxicity through oxidative stress, inflammation, and apoptosis together with induction of genotoxicicty.

5 | CONCLUSION

$CoCl_2$ toxicity should be considered a threat to the public as a result of the health risk it poses to humans and animals. We, therefore, propose the mechanism through which $CoCl_2$ induced hepatic damage might be via oxidative stress, inflammation, and apoptosis. Also, $CoCl_2$ dose-dependently induced genotoxicity.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

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